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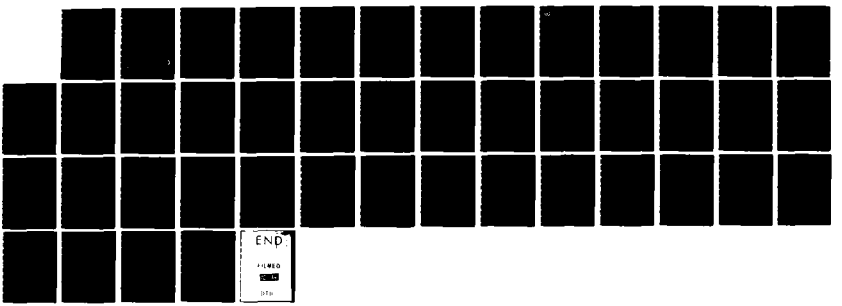

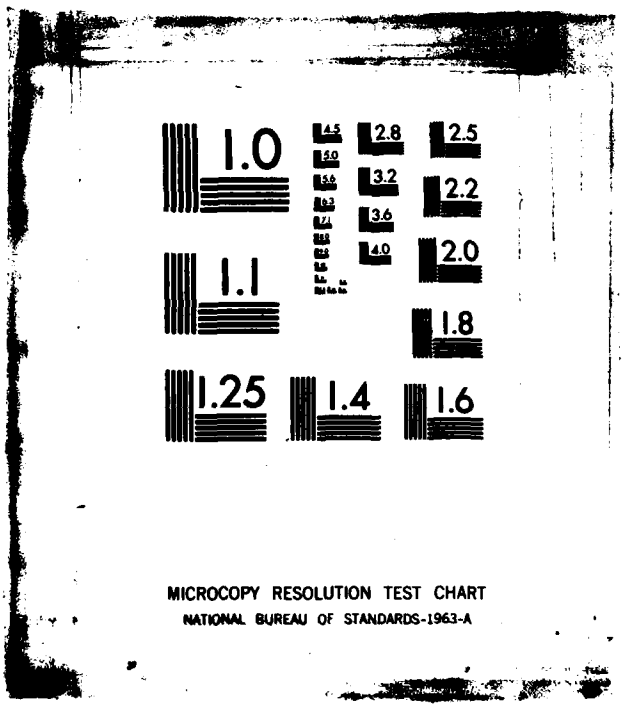
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INSTITUTE REPORT NO. 163

ACUTE DERMAL TOXICITY POTENTIAL OF 1-acetyloctahydro-3, 5 , 7-trinitro-1, 3, 5, 7-tetrazocine (SEX) IN MALE AND FEMALE RABBITS

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CRAIG W. WHITE, DVM, CPT VC
and
GLEN E. MARRS, DVM, MS, MAJ VC

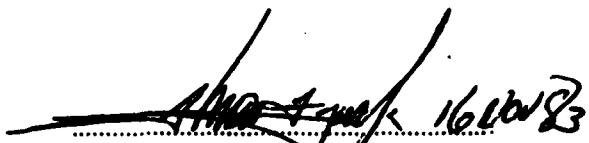
TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

NOVEMBER 1983

Toxicology Series 60

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Acute Dermal Toxicity Potential of 1-Acetyloctahydro-3,5, -Trinitro-1, 3,5,7-Tetrazocine (SEX) in Male and Female Rabbits (Toxicology Series 60)-- Mullen, White, and Marrs



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7. AUTHOR(s): Lawrence Mullen, BS, SP4 Craig W. White, DVM, MAJ VC Glen E. Marrs, DVM, MAJ VC	8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Toxicology Group, Division of Research Support Letterman Army Institute of Research Presidio of San Francisco, CA 94129	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: Project: 612720.835AA
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18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine (SEX), Acute Dermal Toxicity, Holston Army Ammunition Plant, CAS Reg. No. 13980-00-2, Nitramines

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The acute dermal toxicity potential of 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX) was determined in rabbits by using abraded skin sites and plastic covering over the exposed areas for 24 hours. There were no compound-related deaths during this study.

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ABSTRACT

The acute dermal toxicity potential of 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX) was determined in rabbits by using abraded skin sites and plastic covering over the exposed areas for 24 hours. There were no compound related deaths during this study.

KEY WORDS: 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine (SEX), Acute Dermal Toxicity, Holston Army Ammunition Plant, CAS Reg. No. 13980-00-2, Nitramines

PREFACE

TYPE REPORT: Acute Dermal Toxicity GLP Report

TESTING FACILITY: U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

PROJECT/WORK UNIT/APC: 612720.835AA Acute Mammmalian Toxicology Testing
APC TL06

GLP STUDY NUMBER: 82003

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC,
Diplomate, American College of
Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: CPT Craig White, DVM, VC

CO-PRINCIPAL INVESTIGATOR: SP4 Lawrence Mullen, BS

PATHOLOGIST: Glen E. Marrs Jr., DVM, MS, MAJ, VC
Diplomate, American College of
Veterinary Pathologists

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, retired SOPs, raw data, analytical, stability, and purity data of the test compound, tissues, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: 1 Acetyloctahydro-3,5,7-Trinitro-
1,3,5,7-Tetrazocine (SEX)

INCLUSIVE STUDY DATES: 19 January 1983 - 22 February 1983

OBJECTIVE: The purpose of this study was to determine the acute dermal toxicity potential of SEX in rabbits.

ACKNOWLEDGMENTS

The authors wish to thank SP5 Leonard Sauers, MS; SP5 Florence McKinley, BS; SP5 Marlin McKinley, BS; SP5 Thomas Kellner, BA; SP4 Justo Rodriguez, BS; SP5 Evelyn Zimmerman; Carolyn Lewis, MS; Thomas Hironaga; Lucille Cote; and John Dacey for their assistance in performing the research. In addition, we wish to thank Jesse Barkley Jr., US Army Medical Bioengineering Research and Development Laboratory, for his assistance as Project Consultant.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY:

We, the undersigned, believe the study number 82003 described in this report to be scientifically sound and the results in this report and interpretation to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations for Non-Clinical Laboratory Studies, outlined by the Food and Drug Administration.

JOHN T. FRUIN / DATE
COL, VC
Study Director

CRAIG W. WHITE / DATE
CPT, VC
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DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

9 Aug 83

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 82003 the following inspections were made:

7 Feb 83, 0940
7 Feb 83, 1315
13 May 83

The report and raw data for this study were audited on 5 Aug 83.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the April and July 1983 report to management and the Study Director.

NELSON R. POWERS, Ph.D.
CPT, MSC
Quality Assurance Officer

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APPENDICES

Acute Dermal Toxicity Potential of 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine (SEX) in Male and Female Rabbits--Mullen et al

The manufacture of the explosives hexahydro-1,3,5-trinitro- 1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7- tetrazocine (HMX) at the Holston Army Ammunition Plant (HSAAP) results in the formation of a by-product, 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX). It is formed during nitrolysis of hexamine. During this process, a portion of the hexamine is also acetylated by the acetic acid/acetic anhydride solvent. As a result, significant quantities of SEX are discharged from HSAAP. HSAAP is the only known producer of SEX. Its discharge, while partially mitigated by pollution abatement facilities at HSAAP, will continue, and could increase at mobilization. The information on the chemical, physical and toxic properties of SEX is limited. Many of its properties can only be inferred by comparison with RDX and HMX. Although no specific data are available, SEX, based on structural comparisons, appears to be relatively more hydrophilic than either RDX or HMX and thus, potentially, a more serious toxic threat to the aquatic life in the Holston River than RDX or HMX. Letterman Army Institute of Research (LAIR) has been tasked with assessing toxicologic hazards of SEX (1-3). This report summarizes the results of one of the studies in the series.

Description of Test

Methods of testing compounds for their potential irritancy or toxicity have become standardized over the years by the cooperative efforts of the Environmental Protection Agency, Food and Drug Administration, U.S. Consumer Product Safety Commission numerous subcommittees, and the Armed Forces Research departments (4-6).

A test for acute dermal toxicity evaluates the potential for systemic toxic effects of chemicals that contact the skin. This is done by determining the median lethal dose (LD_{50}) of a single dermal exposure to the animal species under test.

Dermal toxicity is one of the three categories of toxicity defined by route of exposure in the Federal Hazardous Substances Act (FHSA). The adult albino rabbit is the preferred species for such reasons as size, ease of handling and restraint, and skin permeability. The animal's dorsal and lateral sections are close clipped so that no less than 10% of the body surface area is available for application of material (7). The abdominal section is not clipped.

The maximum quantity of test substance applied is 2 g/kg. The test dose must remain in contact with the skin throughout the 24-hour exposure period. This is assured by application of the dose inside an impermeable cuff made of plastic film. The cuff or sleeve is constructed so that the ends are reinforced and fit snugly around the trunk of the animal. The ends are tucked to permit the central portion to "balloon" and to furnish a reservoir for the dose. Such devices occlude the skin and thereby enhance penetration and potential toxicity of the test material. For this reason, routine use of occlusive dressing is not recommended unless anticipated human exposure warrants it. For materials of anticipated low toxicity, an initial range-finding dose of 2 g/kg of body weight applied to five or more animals of each sex with abraded skin is sufficient to demonstrate a lack of appreciable dermal toxicity. At the end of the exposure periods, any residual material is gently removed with a gauze compress, the exposed area examined at least daily for signs of systemic toxicity and localized dermal reaction. After the 14-day observation period, animals are sacrificied. A gross necropsy is performed on each rabbit and two sections of its exposed skin are processed for histopathology (8).

Objective of Study

The objective of the study is to determine the acute dermal toxicity potential of 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX) in rabbits.

METHODS

Chemical Analysis

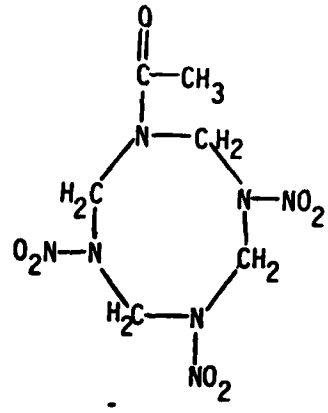
Information on the chemical analysis of SEX appears in Appendix A.

Test Substance

Chemical name: 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine

CAS: (13980-00-2)

Structural formula:



Empirical formula: $C_6H_{11}N_7O_7$

Vehicle: Isotonic sodium chloride (saline) was the only solvent used in this procedure.

Compound Preparation

SEX, 2 g/kg body weight, was weighed into plastic weigh boats (Mettler AK160 Balance, Serial No. A70328,LAIR FSN 6670-C02-5032). The compound was then mixed with 20 ml of isotonic sodium chloride solution to make a paste. The test material was applied uniformly over the prepared dorsal surface area (240 cm^2) or approximately 10% of the body surface and held in contact with the skin by a porous gauze dressing for 24 hours.

Animal Data

A total of 22 (11 males and 11 females) New Zealand White rabbits were received from Elkhorn Rabbitry, Watsonville, CA. Additional animal data appear in Appendix B.

Environmental Conditions

A commercially available certified rabbit chow and tap water were provided ad libitum for the animals during the study. Appendix C is a complete listing of the environmental conditions of this study.

Dosing

Dosing Levels: The test was conducted as a limit test (SOP-OP-STX-30) wherein 5 males and 5 females are assigned to the test group receiving 2 g/kg SEX. A concurrent vehicle control group of 5 males and 5 females was tested. The only solvent/moistening agent to be used in this procedure was isotonic sodium chloride (U.S.P.). Each control animal received 20 ml of isotonic sodium chloride (U.S.P.). If a test is conducted at this dose level and no test compound-related

mortality occurs, then a full study using 3 dose levels is not necessary (1). For a standard test, 10 animals per dose group would have been used - one half of these animals would have the exposed area abraded and the other half would remain intact (6).

Dose Volume: According to body weight; range 4.99 to 5.73 g of test compound mixed with 20 ml of saline to make a paste.

Duration of Exposure: 24 hours.

Method and Frequency of Administration: The application sites in all animals were abraded by use of an abrading tool designed for this experiment (9). It has four small metal points mounted onto a flat piece of metal that is attached to a handle. This instrument was drawn across the area to be exposed so that only the stratum corneum was disrupted. The lines were approximately 2 cm apart along the axis of the backbone over the entire exposed surface. The test material was mixed with 20 ml of isotonic sodium chloride (USP) to make a paste. The test material was applied uniformly over the prepared dorsal surface area and held in contact with the skin by a porous gauze dressing. The dorsal and abdominal areas were then covered with plastic wrap (5 mm polyethylene) derived from GSA bags (#NSN 8105-00-655-8285) and taped on the ends and seam with Conform® adhesive tape (Kendal Hospital Products, Boston, MA 02110, Code No. 7233). The animals were observed and clinical signs recorded within six hours of administration of the test material. The bandage was removed after 24 hours. All residual material was removed by washing with saline and then wiping the animals with gauze pads.

Observations

Animals were weighed seven times over the study test period. Observations were recorded three times on the day of dosing and twice a day for the remainder of the study.

When data were recorded, location, area, and intensity were graded according to a scale at the side of the data sheets. This scale includes five parameters to define area and severity. Area is defined as $\leq$ 5%, $\leq$10%, $\leq$25%, $\leq$50%, and > 50% while severity is defined as very slight, slight, moderate, well-defined, and severe. Examples of dermal irritation include erythema, edema, blister, necrosis, and pitting. Thus an observation would describe erythema as very slight, involving an area $\leq$10% and occurring on the back. At the end of the 15-day period, animals were anesthetized with sodium pentobarbital, sacrificed by exsanguination from severed axillary vessels and evaluated at necropsy. Skin was taken from an abraded and non-abraded area and examined microscopically.

Duration of Study

The study period was 15 days with a 25-day quarantine/acclimation period. Historical study events are listed in Appendix D.

Changes in the Original Procedures or Protocol During the Study

Rabbits were reclipped the morning of dosing to assure adequate area exposed.

Approximately one-half hour after dosing, animals 83F11, 83F15, and 83F19 had to be rewrapped.

Rabbits were observed for fifteen days after dosing.

Surgical adhesive tape (DLA 120-82-C-4463, Chaston Medical and Surgical Products, Dayville, CT 06241) was used to tape the gauze pads to all of the rabbits on this study. This procedure caused irritation in the neck area of several animals. However, the tape irritation did not effect the dermal response to SEX.

RESULTS

Clinical Observations

During the course of the study, observations were split into two major categories - those that applied to the general health of the animal and those which were related to skin exposure.

Systemic: No clinical systemic signs were noted that were interpreted as signs of toxicity attributable to the test compound. Two of five female animals in both the control group and the SEX-dosed group demonstrated clipper burns during the course of the study. Additionally, one of five females in the control group and three of five females in the SEX-dosed group demonstrated tape irritation on the neck. Two of five males in the control group demonstrated clipper burns, and three of five males in the control group had tape irritation. For the SEX-dosed group, one of five males had a clipper burn and one of five males had a tape irritation.

Dermal: Skin irritation scores at 24 hours, 48 hours, and 72 hours for erythema and edema are presented in Appendix E.

The most notable signs related to dermal toxicity were erythema, edema, scaling, and scabbing. Figures 1 to 4 (Appendix F) show the average duration of clinical signs in male and female rabbits.

Vehicle Controls

Males

Erythema was seen in four of five control males. The location, area, and intensity were graded as described in methods. Very slight erythema occurred on the back and along the abrasions involving a maximum area of $\leq$ 5%. These rabbits exhibited erythema for an average of one day.

Scaling occurred in two of five control males. Scaling was noted on the back and abraded areas; the maximum area was $\leq$ 5% and the maximum intensity was very slight. The average duration was approximately three days.

One of five control group males had very slight edema occurring on the back involving a maximum area of $\leq$ 5%. One of five control group males had a slight lesion on the back that involved a maximum area of $\leq$ 5%.

Additionally, one of five males in the control group had a very slight scratch on the abraded area. The scratch involved a maximum area of $\leq$ 5%. The edema and lesion persisted for one day while the scratching occurred for three days.

Females

Erythema occurred in five of five control group females. The maximum severity was moderate and the maximum area involved was $\leq$ 10%. The erythema occurred on the back and the abraded areas. The average duration of erythema was approximately four days.

Three of five control females developed scaling. The scaling was very slight in severity occurring on the abraded area and also the right hind leg; it involved a maximum area of $\leq$ 5%. The average duration of scaling was approximately five days.

Very slight edema, involving a maximum area of $\leq$ 5%, occurred in two of five females in the control group. The edema occurred on the back and abraded areas and persisted an average of three days.

Very slight scab formation on the back and abraded areas involving a maximum area of $\leq$ 5% occurred in two of five females in the control group. The average duration of scab formation was approximately six days.

SEX-Dosed Rabbits:

Males

Two of five males in the SEX-dosed group demonstrated erythema. The erythema was located on the back and the left hind leg. The maximum severity was slight; the maximum area involved was $\leq$ 5%. The average duration for erythema was approximately three days.

Scaling occurred in one of five SEX-dosed males. Scaling was observed on the back, and the maximum severity was slight. The maximum area was $\leq$ 5%. The duration of scaling was three days.

Very slight edema occurred on the back of one of five males in the SEX-dosed group. The maximum intensity was $\leq$ 5%. Edema persisted for approximately two days.

Scab formation was observed on the back and left flank area of one of five SEX-dosed males. The maximum severity for scab formation was slight; it involved a maximum area of $\leq$ 5%. Scab formation was noted for approximately four days.

Females

Three of five females in the SEX-dosed group had erythema. The erythema was observed on the back and abraded areas. The maximum severity was slight and the maximum area was $\leq$ 25%. The average duration of erythema was approximately two days.

Three of five females in the SEX-dosed group exhibited scaling. The maximum severity was slight. The maximum area was $\leq$ 5%. Scaling occurred on the abraded areas, right flank, back, right hind leg, and the right lateral section. The average duration of scaling was approximately five days.

Slight and very slight scratching occurred in three of five SEX-dosed females. The maximum area was $\leq$ 5%. The scratching was on the abraded areas only, which persisted an average of approximately three days.

Scab formation on the back, neck, left and right hind legs was noted in three of five SEX-dosed females. The maximum severity for scab formation was slight, while the maximum area was $\leq$ 5%. Scab formation lasted an average of three days.

Treatment of Animal Diseases and Injury

Rabbits were placed on therapeutic levels of sulfaquinoline (3.2 ml per 236 ml bottle) of drinking water for coccidiosis prophylaxis during quarantine. They did not receive sulfaquinoline after they were placed in the GLP suite.

Gross Pathological Observations

It does not appear that the application of SEX to close-clipped abraded skin of male and female rabbits for 24 hours caused or intensified the inflammatory response that could be detected 14 days after application. A report of gross pathological observations appears in Appendix G.

DISCUSSION

There were no deaths for rabbits dosed at 2 g /kg body weight during the acute dermal toxicity test. The acute dermal toxicity test also revealed that SEX did not cause clinical signs of systemic toxicity when applied to approximately 10% of the rabbits' body surface. SEX did produce a slight dermal irritation; however, the saline vehicle control group animals, exhibited a similar dermal response. This lack of a differential dermal response in the experimental group versus the control group may represent the toxic potential of SEX. Conversely, it could be a function of the hydrophobic nature of SEX which prohibits one from obtaining "effective" dermal concentrations of SEX even when it is administered in a saline paste.

CONCLUSION

SEX caused only a slight dermal irritation to the clipped skin of rabbits exposed for a 24-hour period and observed for fifteen days. However, a similar response was observed in the vehicle control group. Therefore, it can be concluded that SEX produces minimal dermal toxicity under the conditions of this study.

RECOMMENDATION

Additional toxicological evaluations of SEX, if required, should be conducted. Protocols should be written which take into account the insolubility of SEX in standard vehicles.

REFERENCES

1. Tyson CA, Dilley JV, Sasmore DP, Spanggord RJ, Newell GW, Dacre JC. Single-dose and repeated-exposure toxicity of a complex wastewater from munitions manufacturing plants. J Toxicol Environ Health 1982;9:545-564.

2. Dilley JV, Tyson CA, Spanggord RJ, Sasmore DP, Newell GW, Dacre JC. Short-term oral toxicity of a 2,4,6-trinitrotoluene and hexahydro-1,3,5-trinitro 1,3,5-triazine mixture in mice, rats, and Dogs. J Toxicol Environ Health 1982; 9:587-610.

3. Bedford CD, Deas BD, Broussard MM, Geigl MA, Marynowski CW. Preparation and purification of multigram quanitities of TAX and SEX. Third Phase, Final Report. Fort Detrick, Maryland: US Army Medical Research and Development Command, December 1981.

4. Environmental Protection Agency. Good Laboratory Practices proposed regulations (40 CFR 770,771,772) and preamble as published in the Federal Register, 22 Aug 78, 9 May 79, 26 Jul and 18 Apr 80, (45 FR26373).

5. Food and Drug Administration. Good Laboratory Practices regulations (21 CFR58) and preamble, published in the Federal Register, 22 Dec 78 (45 FR59986-60025).

6. Interagency Regulatory Liaison Group Testing Standards and Guidelines Work Group. Recommended guideline for acute dermal toxicity test. 1981.

7. Association of Food and Drug Officials of U.S. Appraisal of the safety of chemicals in foods, drugs and cosmetics (4th printing). 1959.

8. Committee for the Revision of NAS Publication 1138. Commmittee on Toxicology, National Research Council. Principles and procedures for evaluating the toxicity of household substances. Prepared for the Consumer Product Safety Commission. Washington, DC: National Academy of Sciences, 1977.

9. Haley TJ, Hunziker J. Instrument for producing standardized skin abrasion. J Pharmaceut Sci 1974; 63:106.

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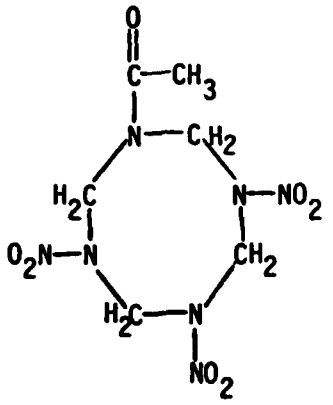
APPENDICES

CHEMICAL DATA

Chemical name: 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine

Chemical Abstract Service Registry No.: 13980-00-2

Structural formula:



Empirical formmula: $C_6H_{11}N_7O_7$

Molecular weight: 293.2 g/mole (calculated). Typical experimental values Rast camphor method - 207 to 377 g/mole.

Physical state: Solid at 20 C

Melting Point: 224.2 - 224.7 C

Density: 1.785 g/cc at 21 C

ph: N/A nonaqueous

Compound Refractory Index: Unknown

Stability: Unknown

Purity: 99.9%

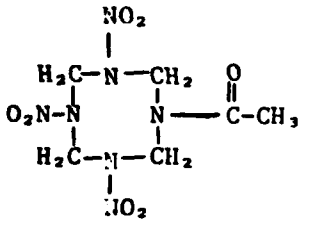
Manufacturer: SRI International
Menlo Park, CA 94205

FROM: Preparation and Purification of Multigram Quanitites of TAX and SEX, Bedford *et al*, Organic Chemistry Department, SRI International, 333 Ravenwood Avenue, Menlo Park, CA 94025

CHARACTERIZATION OF SEX

SEX appears sufficiently stable in normal nitrolysis media to exist as a contaminant in RDX/HMX manufacturing process. The characteristics of SEX are as follows:

Structural Formula:



Empirical Formula: $C_6H_{11}N_7O_7$

Elemental Analysis: Calculated: C, 24.57; H, 3.75; N, 33.45
C, 24.21; H, 3.76; N, 33.45

Melting Point: 237°-237.5°C (DEC)

Density: 1.785 g/cm^3 at 21°C

Molecular Weight: 293 (Calculated)

Solubility: Soluble in dimethylsulfoxide. Slightly soluble in acetone, nitromethane, and acetonitrile. Almost insoluble in ethanol, benzene, and ether.

Impact Sensitivity (drop weight test): Greater than 300 kg-cm compared with 148 kg-cm for pure HMX. SEX is sensitive to direct strong hammer blows. During our investigations SEX has exhibited no instability, but because of the hammer results should be handled as a potential explosive, like HMX.

Infrared Spectrum: See Figure 7.

Proton NMR Spectrum: See Figure 8.

Chemical Properties: SEX gives a positive Franchimont nitramine reaction, but a negative Liebermann nitroso test. Decomposition in hydroxide fails to produce free CH_3COO^- for a lanthanum nitrate test.

However, if SEX is decomposed in 96% sulfuric acid, the distillate gives a lanthanum nitrate test.[7]

SEX appears inert to boiling acetic anhydride and unaffected by treatment with ammonium nitrate-nitric acid mixtures. Absolute nitric acid at 50°-60°C converts SEX to HMX. Warm 70% nitric acid destroys the compound rapidly, as does 10% aqueous sodium hydroxide and 28% ammonia.

Purity: The purity of SEX was determined by analytical HPLC with a Spectra-Physics 3500B Liquid Chromatograph. A waters RCM-100, C_{18} cartridge with a mobile phase of 80/20 water/methanol was used for DADN/SEX/HMX mixtures. An internal standard of RDX was used with $1/R_f$* values of 1.5 for HMX, 1.5 for SEX, and 1.7 for DADN. Hot-column chromatographed SEX contained no detectable amounts of DADN (starting material) and only 1% to 2% HMX (sole contaminant). High pressure liquid chromatographed material contained no DADN or HMX. Also, no other contaminants were detected by analytical HPLC, ensuring a 99.9+% purity of SEX.

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*R_f = response factor.

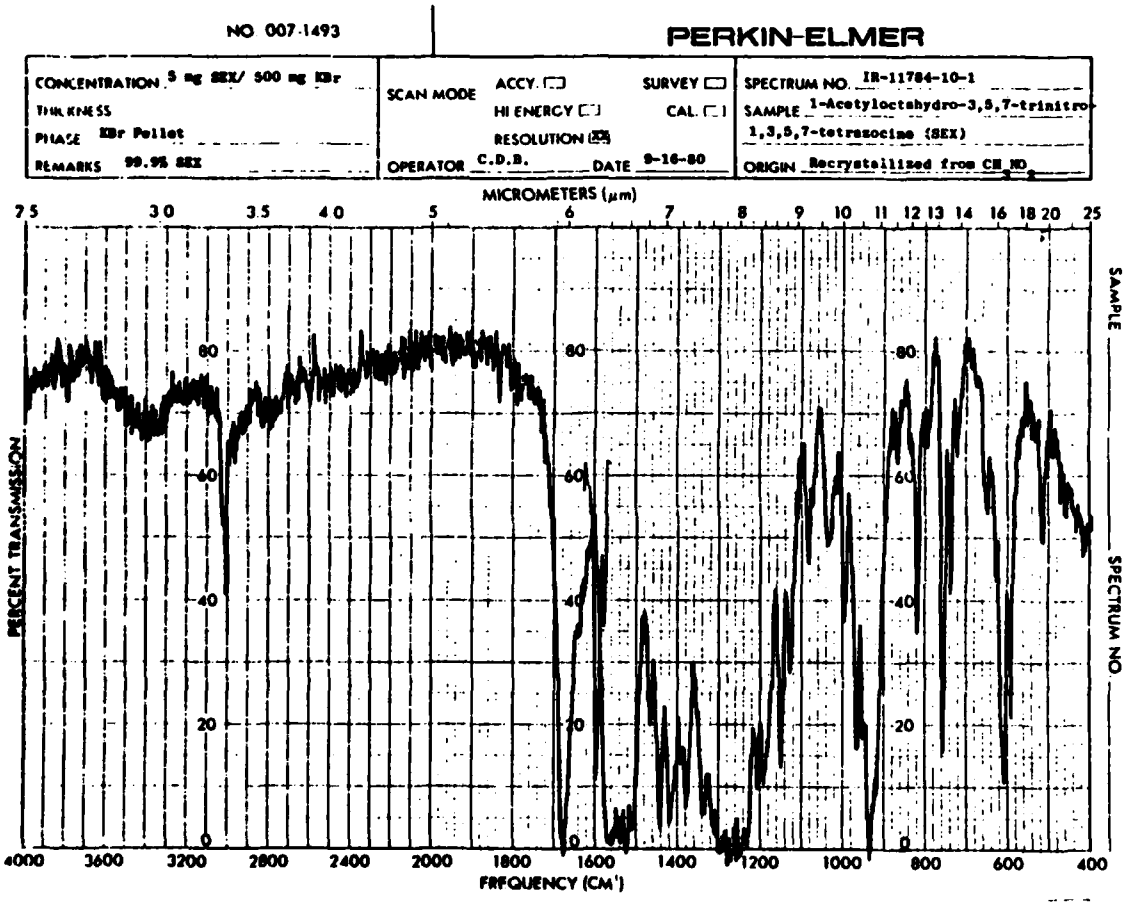


FIGURE 1 INFRARED SPECTRUM OF 99.9+ % SEX

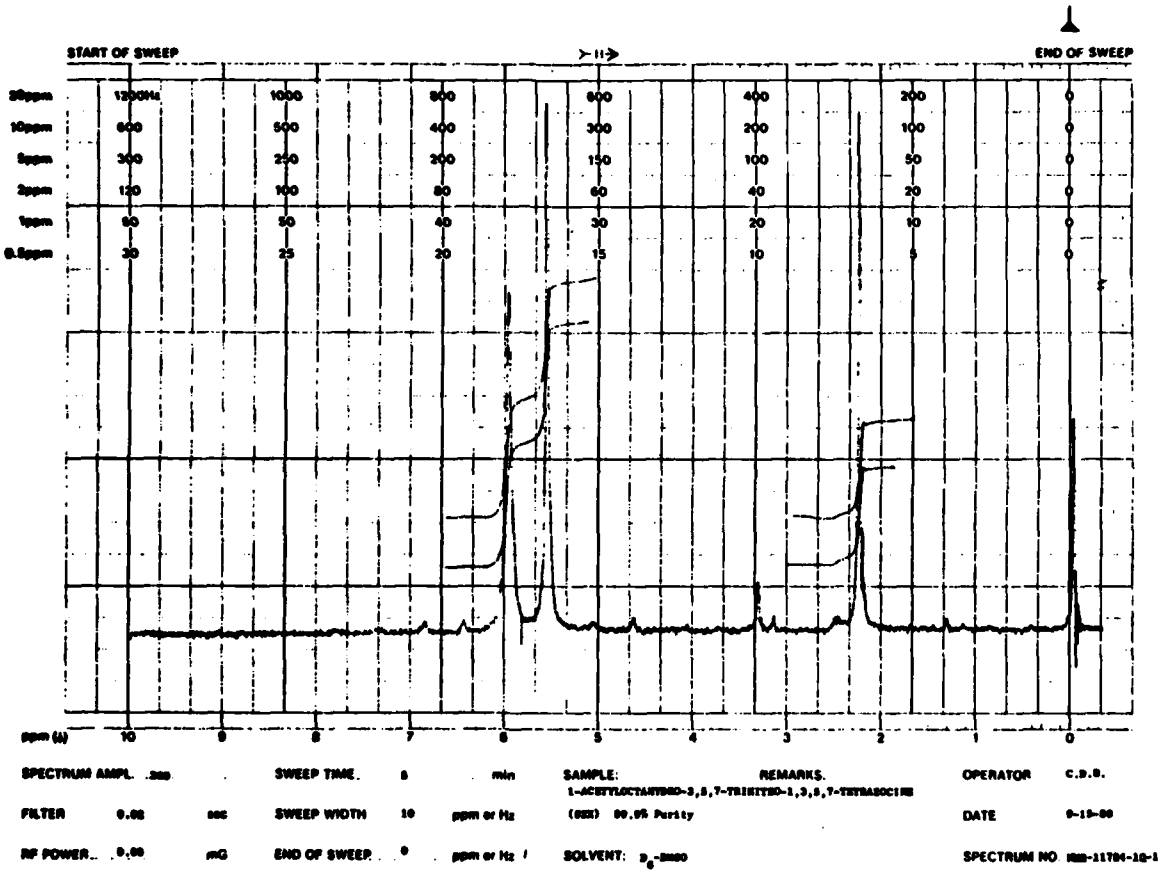


FIGURE 2 PROTON NMR SPECTRUM OF 99.9+ % SEX

APPENDIX A (concluded)

ANIMAL DATA

Species: Rabbit

Strain: New Zealand White

Rationale for selection: The New Zealand White Rabbit is a proven mammalian model for acute dermal studies because of its size, ease of handling, restraint, and skin permeability.

Source: Elkhorn Rabbitry
565 Starr Way
Watsonville, CA 95076

Pretest Conditioning:

a. Arrival at LAIR 13 Jan 83, quarantine time 18 days.

b. Animals clipped the day before dosing.

c. Animals given sulfaquinoline (SQ) during quarantine, at a standard dosage of 3.2 ml SQ per 236 ml water ad lib for seven days.

Restraint: Manual restraint during application. After dosing, the animals were placed in their cages; the bandages were not disturbed over the 24-hour period.

Sex: Male and female.

Age: Young adult

Method of Randomization: Manually by Random Numbers Table

Animals in Each Group: 5 males and 5 females per test chemical; 5 males and 5 females in wrapped saline control.

Condition of Animals at Start of Study: Normal

Mean Weight ($\pm$ 1 standard deviation) at Dosing:
2752.4 ($\pm$ 119)g for test animals
2853.1 ($\pm$ 134)g for control animals

Mean Weight ($\pm$ 1 standard deviation) at Sacrifice:
2818.6 ($\pm$ 118) g for test animals
2886.0 ($\pm$ 131) g for control animals

Identification Procedures: Ear tattooed IAW SOP OP-ARG-1

ENVIRONMENTAL CONDITIONS

Caging: Number/cage = 1; type used = stainless steel, wire mesh bottom, battery type, no bedding.

Diet: Certified Ralston Purina Rabbit Chow 5322

Water: Central line to cage battery.

Temperature: 76 ± 5 F (24 ± 3 C) degrees

Relative humidity: 45 ± 5%

Photoperiod: 0600-2000 h/day (light 14 h).

APPENDIX C

HISTORICAL LISTING OF STUDY EVENTS

Date	Event
13 Jan 83	Male and female rabbits arrived at LAIR. They were checked for illness and quarantined in Room RS1409.
31 Jan 83	10 males and 10 females were removed from quarantine, separated into test groups and prepared for study
2 Feb 83	Hair was clipped from the back and sides.
7 Feb 83	Rabbits were dosed according to SOP-OP-STX-30. The clipped areas were abraded and test substance applied. Rabbits were observed frequently after dosing. Clinical signs were recorded three times after dosing.
8 Feb 83	Bandaging materials were removed. Animals were observed.
8-21 Feb 83	Clinical observations were recorded twice a day.
22 Feb 83	Animals were not fed; euthanasia and necropsies were performed, and several sites selected for histopathological observation.

APPENDIX D

LIST OF TABLES

APPENDIX E

GLP Study No. 82003

Dose Date: 7 February 1983 Test Substance: (SEX)

TABLE 1
ACUTE DERMAL TOXICITY
IRRITATION SCORES FOR SKIN (24h, 48h, 72h)

	Erythema			Edema		
Male Animal No.	24h	48h	72h	24h	48h	72h
83F13	0	0	0	0	0	0
83F14	0	0	0	0	0	0
83F17	0	0	1	0	0	1
83F18	0	0	0	0	0	0
83F20	0	0	0	0	0	0
Female Animal No.						
83F01	1	1	1	0	0	0
83F05	2	0	0	0	0	0
83F06	1	0	0	0	0	0
83F07	0	0	0	0	0	0
83F09	0	0	0	0	0	0

Erythema Formation:

	Value
None	0
Very slight	1
Well-defined	2
Moderate	3
Severe	4

Edema Formation:

	Value
None	0
Very slight	1
Slight	2
Moderate	3
Severe	4

Initial/Date: 11 May 1983 Lawrence Mullen

GLP Study No. 82003

Dose Date: 7 February 1983 Test Substance: (USP) Control Saline

TABLE 2
ACUTE DERMAL TOXICITY
IRRITATION SCORES FOR SKIN (24h, 48h, 72h)

	Erythema			Edema		
Male						
Animal No.	24h	48h	72h	24h	48h	72h
83F11	1	0	0	0	0	0
83F12	0	0	0	0	0	0
83F15	1	0	0	0	0	0
83F16	0	0	0	0	0	0
83F19	1	0	0	0	0	0
Female						
Animal No.						
83F02	1	1	1	0	0	1
83F03	1	1	1	0	0	1
83F04	2	2	0	0	0	0
83F08	1	0	0	0	0	0
83F10	1	0	0	0	0	0

Erythema Formation:

	Value
None	0
Very slight	1
Well-defined	2
Moderate	3
Severe	4

Edema Formation:

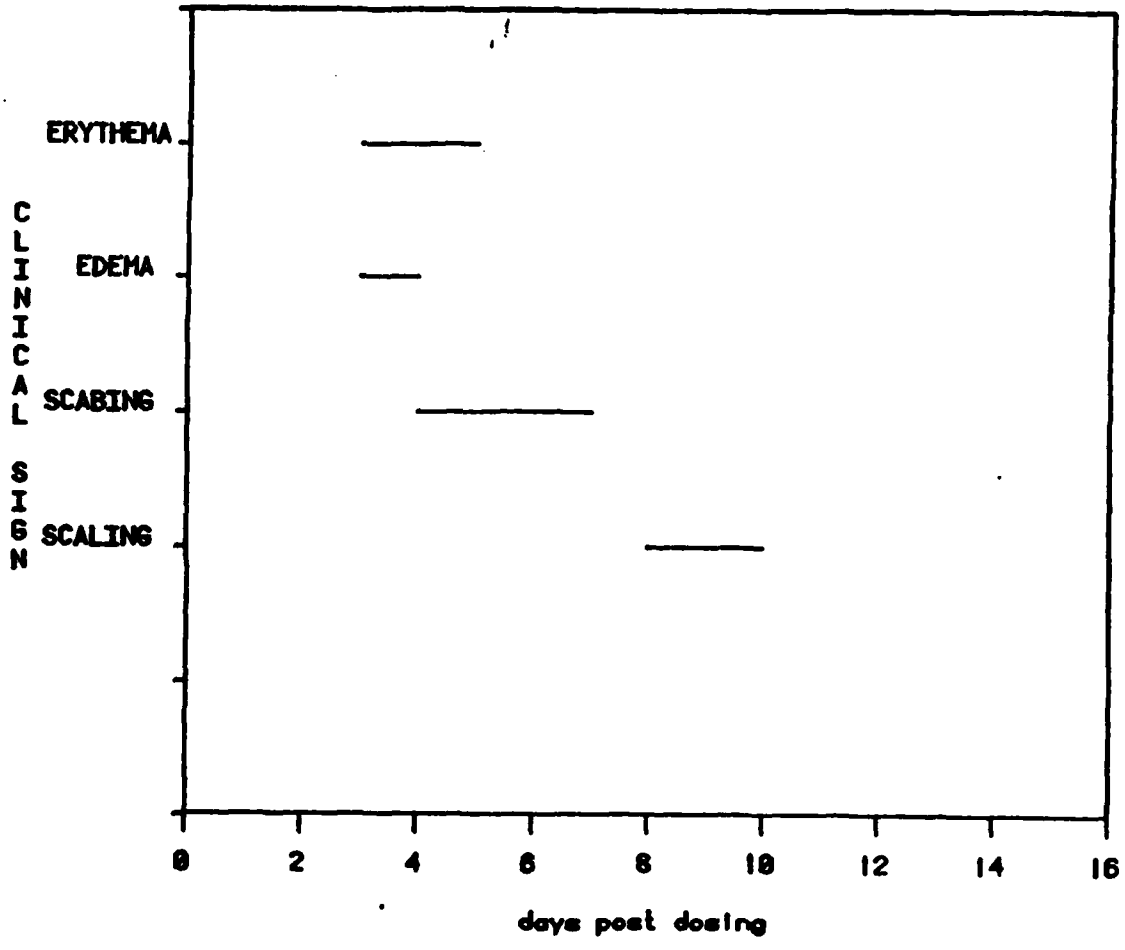
	Value
None	0
Very slight	1
Slight	2
Moderate	3
Severe	4

Initial/Date: 11 May 1983 Lawrence Mullen

LIST OF FIGURES

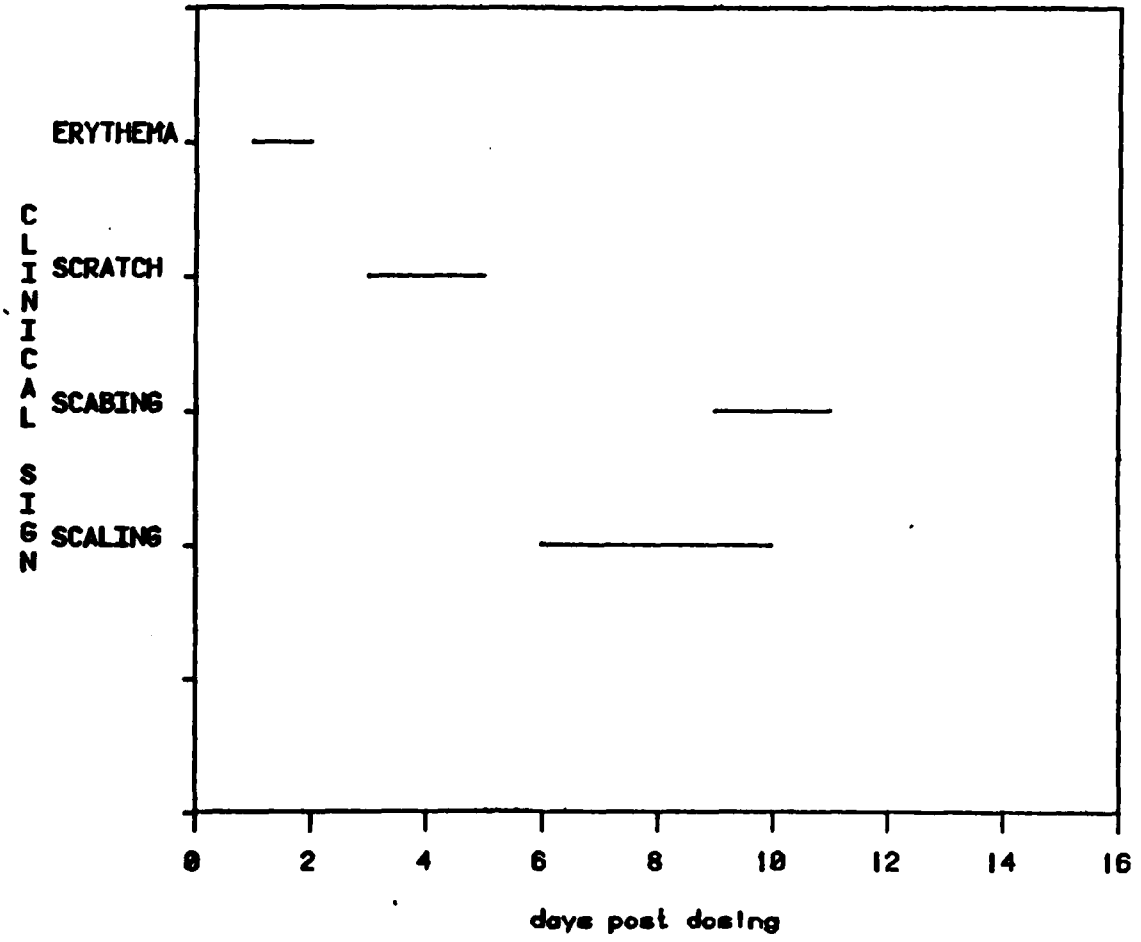
APPENDIX F

FIGURE 1
ACUTE DERMAL TOXICITY OF SEX- GLP STUDY 82003
AVERAGE DURATION OF CLINICAL SIGNS IN DOSED MALE RABBITS



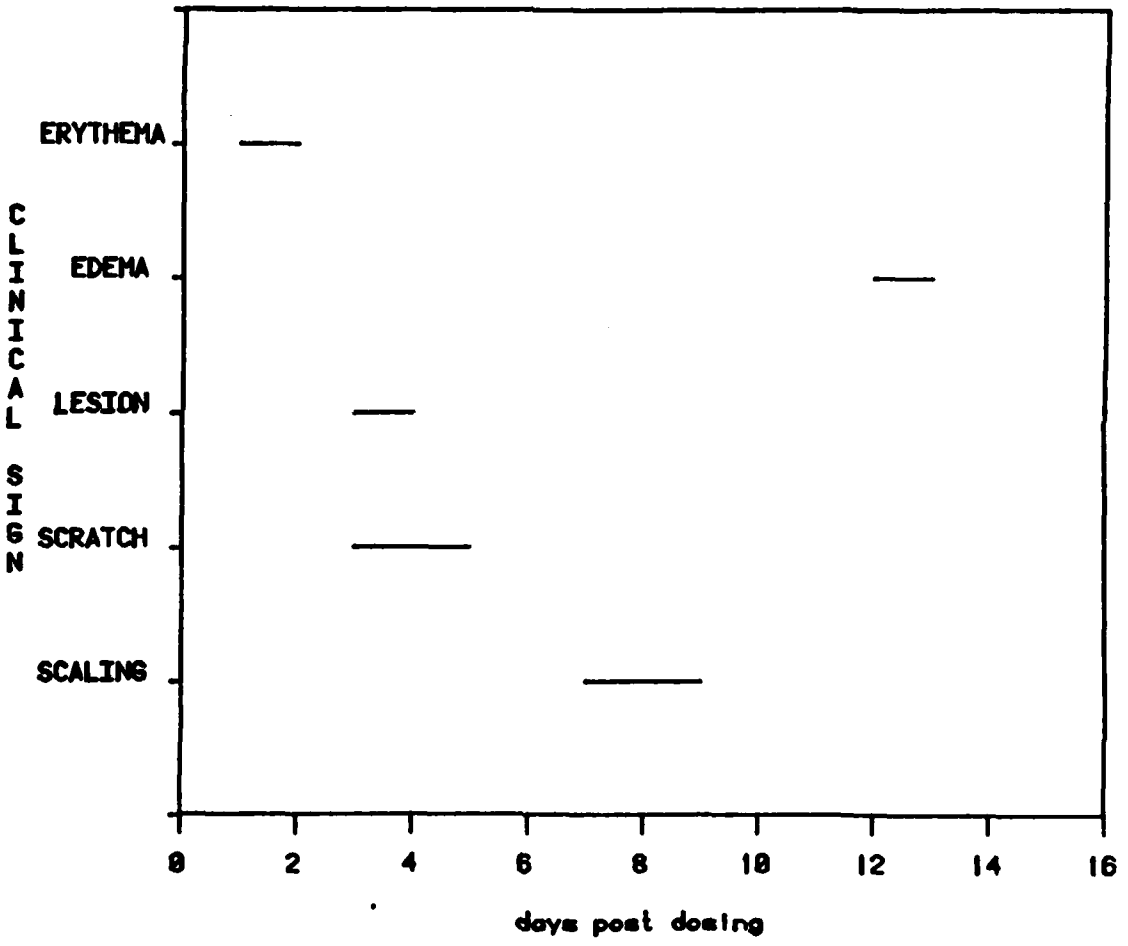
APPENDIX F (cont.)

FIGURE 2
ACUTE DERMAL TOXICITY OF SEX- GLP STUDY 82003
AVERAGE DURATION OF CLINICAL SIGNS IN DOSED FEMALE RABBITS



APPENDIX F (cont.)

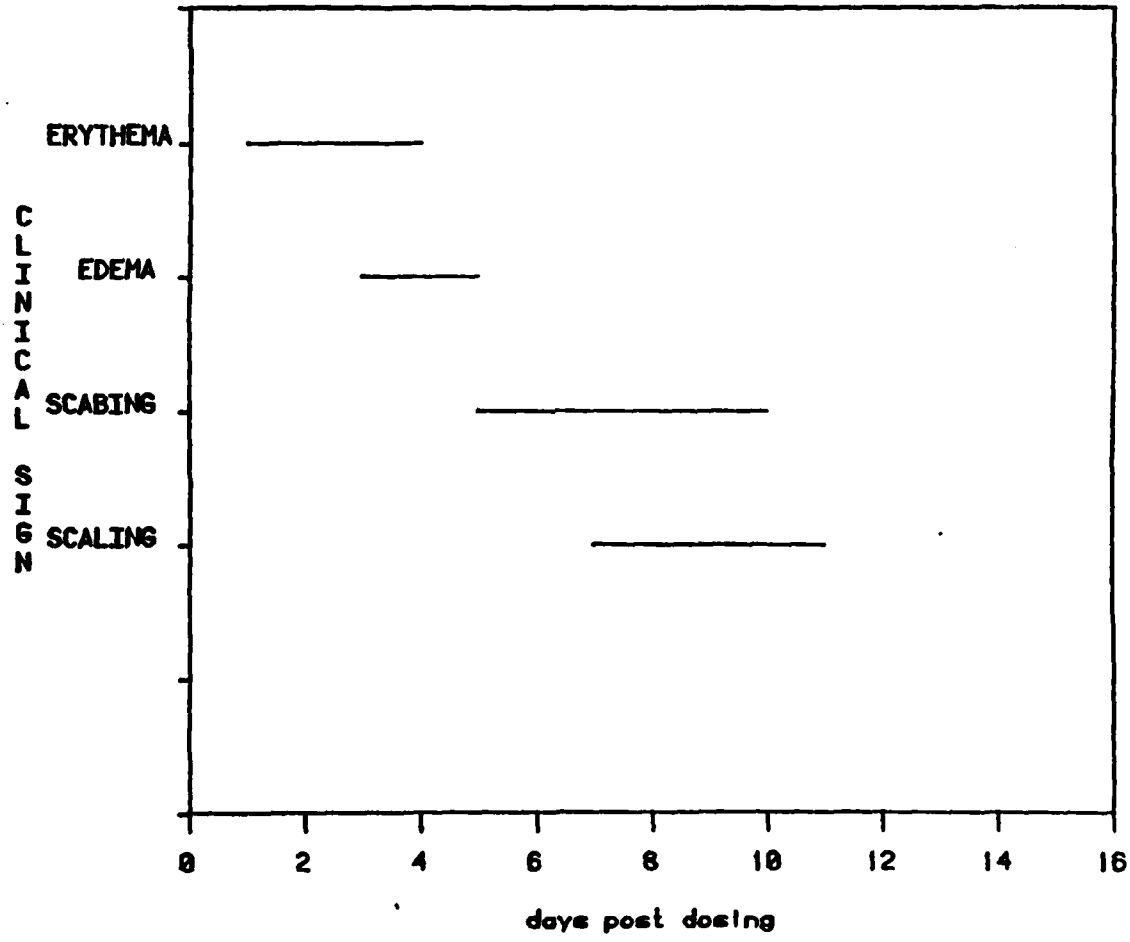
FIGURE 3
GLP STUDY 82003 - ACUTE DERMAL TOXICITY OF SEX
AVERAGE DURATION OF CLINICAL SIGNS IN CONTROL MALE RABBITS



APPENDIX F (cont.)

FIGURE 4

ACUTE DERMAL TOXICITY OF SEX- GLP STUDY 82003
AVERAGE DURATION OF CLINICAL SIGNS IN CONTROL FEMALE RABBITS



APPENDIX F (concluded)

PATHOLOGY REPORT

GLP Study 82003

Acute Dermal Toxicity Study Test (LD_{50}) in Male and Female Rabbits of Octahydro-1-(N)-acetyl-3,5,7-trinitro-1,3,5,7-tetrazine (SEX), (CAS #13980-00-2) - Phase I (Limit Test)

History: The purpose of this study was to determine the acute dermal toxicity of SEX in male and female New Zealand White rabbits. Two g/kg of tested material was applied to the clipped and abraded skin of rabbits in Group 2 for 24 hours. Isotonic sodium chloride (U.S.P.) was applied to the clipped and abraded skin of rabbits (saline controls) in Group 1 for 24 hours.

After a 14-day observation period, the rabbits were submitted for necropsy. All rabbits survived until termination of the study. They were killed by exsanguination from severed axillary vessels while under anesthesia produced by intravenous injection of pentobarbital. Complete gross necropsies were performed and two specimens of skin from each exposed area were fixed in neutral buffered formalin, embedded in paraffin, sectioned at approximately 6 micrometers, and stained with hematoxylin and eosin for microscopic examination.

Gross necropsy findings: No gross lesions due to the isotonic sodium chloride (U.S.P). or the SEX were observed in any of the rabbits at the termination of this study. One of 5 males in Group 2 had multifocal pitting of both kidneys that was most likely due to infection with Encephalitozoon cuniculi. One of 5 females in Group 1 had otitis externa and otitis media that was due to ear mites and secondary bacterial infection. One of 5 females in Group 1 and 1/5* females in Group 2 had multifocal areas of erythema in the clipped skin that was due to the clipping procedure prior to necropsy.

Microscopic findings: Five types of microscopic lesions were observed in the rabbit skin from the clipped and abraded sites. The most common type lesion was a minimal to mild, focal, multifocal, or diffuse infiltration of macrophages, lymphocytes, and plasma cells (collectively referred to as mononuclear inflammatory cells) in the upper 15% of the dermis, immediately beneath the epidermis. The second most common lesion was a minimal to mild, focal, multifocal, or diffuse proliferation of fibroblasts and increased amounts of collagen (referred to as fibrosis) that was restricted to the superficial dermis. The third most common lesion was a minimal to moderate, focal, focally extensive, or multifocal epidermal hyperplasia. The epidermis in this foci is 2 - 3 times the thickness for the more

*Number of rabbits affected/Number of rabbits exposed

normal epidermis. Pyonecrotizing dermatitis with ulceration was present in 1 female rabbit in the control group and folliculitis with furunculosis was present in 1 female rabbit in the exposed group. Microscopic findings in each skin section, by sex and experimental group are tabulated in Table I. Table II is a summary of the incidence of skin lesions by sex and experimental group.

Mononuclear inflammatory cell infiltration was present in the superficial dermis of 2/5 male and 5/5 female rabbits exposed to SEX and 4/5 male and 5/5 female control rabbits. Dermal fibrosis was present in 4/5 male and 4/5 female rabbits exposed to SEX and 1/5 male and 3/5 female control rabbits. Epidermal hyperplasia was present in 4/5 male and 4/5 female rabbits exposed to SEX and 0/5 male and 4/5 female control rabbits. Pyonecrotizing dermatitis with ulceration was present in 1/5 female control rabbits and folliculitis with furunculosis was present in 1/5 female rabbits exposed to SEX. Normal skin was present in 1/5 male control rabbits.

Even though dermal fibrosis was present in only 1/5 male controls and epidermal hyperplasia was not present in any of the male controls, the overall incidence and severity of the mononuclear inflammatory cell infiltration, the dermal fibrosis, and the epidermal hyperplasia in male and female rabbits suggests that these lesions are most likely due to the abrasion of the skin and/or clipping of the hair. The dermatitis with ulceration and the folliculitis with furunculosis are either incidental findings or are due to the abrasion of the skin and/or clipping of the hair.

In summary, it does not appear that the application of SEX to close clipped abraded skin of male and female rabbits for 24 hours caused or intensified the inflammatory response that could be detected 14 days after application.

Glen E. Marrs, Jr.

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MAJ, VC
Assistant Chief, Pathology Services Group
Division of Research Support

7 April 1983

GLP Study 82003
Acute Dermal Toxicity Study Test (LD_{50}) in Male and Female Rabbits of SEX
Phase I (Limit Test)

TABLE I

Group I - Male Rabbits (saline controls)

Animal#	Pathology Accession#	Microscopic Findings
83F00011	33469-1	Fibrosis, focal, minimal
	33469-2	Mononuclear inflammatory cell infiltrate, focal, minimal
83F00012	33470-1	Essentially normal skin
	33470-2	Essentially normal skin
83F00015	33473-1	Mononuclear inflammatory cell infiltrate, focal, minimal
	33473-2	Mononuclear inflammatory cell infiltrate, multifocal, minimal
83F00016	33474-1	Essentially normal skin
	33474-2	Mononuclear inflammatory cell infiltrate, focal, minimal
83F00019	33477-1	Mononuclear inflammatory cell infiltrate, multifocal, minimal
	33477-2	Essentially normal skin

Group II - Male Rabbits (2 mg/kg SEX)

83F00013	33471-1	Essentially normal skin
	33471-2	Fibrosis, focal, minimal
		Epidermal hyperplasia, focal, minimal
83F00014	33472-1	Mononuclear inflammatory cell infiltrate, multifocal, minimal
	33472-2	Mononuclear inflammatory cell infiltrate, multifocal, minimal
		Fibrosis, focal, mild
83F00017	33475-1	Epidermal hyperplasia, focal, minimal
	33475-2	Essentially normal skin
83F00018	33476-1	Mononuclear inflammatory cell infiltrate, multifocal, minimal
		Epidermal hyperplasia, focal, minimal
	33476-2	Mononuclear inflammatory cell infiltrate, multifocal, minimal
		Fibrosis, focal, minimal
		Epidermal hyperplasia, focal, minimal
83F00020	33478-1	Fibrosis, focal, minimal
		Epidermal hyperplasia, focal, minimal
	33478-2	Fibrosis, focal, minimal
		Epidermal hyperplasia, focal, minimal

GLP Study 82003
Acute Dermal Toxicity Study Test (LD_{50}) in Male and Female Rabbits of SEX
Phase I (Limit Test)

TABLE I (continued)

Group I - Female Rabbits (saline controls)

Animal#	Pathology Accession#	Microscopic Findings
83F00002	33460-1	Mononuclear inflammatory cell infiltrate, multifocal, minimal Fibrosis, multifocal, minimal Epidermal hyperplasia, multifocal, minimal
	33460-2	Mononuclear inflammatory cell infiltrate, multifocal, mild Fibrosis, multifocal, minimal to mild Epidermal hyperplasia, multifocal, minimal
83F00003	33461-1	Dermatitis, pyonecrotizing, subacute, focal, mild with ulceration Mononuclear inflammatory cell infiltrate, multifocal, minimal Fibrosis, focal, minimal Epidermal hyperplasia, focal, minimal
	33461-2	Mononuclear inflammatory cell infiltrate, multifocal, minimal Fibrosis, multifocal, minimal Epidermal hyperplasia, multifocal, minimal
83F00004	33462-1	Essentially normal skin
	33462-2	Mononuclear inflammatory cell infiltrate, multifocal, minimal Fibrosis, multifocal, minimal Epidermal hyperplasia, multifocal, minimal
83F00008	33466-1	Mononuclear inflammatory cell infiltrate, multifocal, minimal
	33466-2	Mononuclear inflammatory cell infiltrate, multifocal to diffuse, minimal Epidermal hyperplasia, focal, mild
83F00010	33468-1	Mononuclear inflammatory cell infiltrate, multifocal, minimal
	33468-2	Mononuclear inflammatory cell infiltrate, focal, minimal

GLP Study 82003
Acute Dermal Toxicity Study Test (LD_{50}) in Male and Female Rabbits of Sex
Phase I - (Limit Test)

TABLE I (continued)

Group II - Female Rabbits (2 mg/kg SEX)

Animal#	Pathology Accession#	Microscopic Findings
83F00001	33459-1	Mononuclear inflammatory cell infiltrate, diffuse, minimal
	33459-2	Mononuclear inflammatory cell infiltrate, diffuse, minimal Fibrosis, multifocal, minimal Epidermal hyperplasia, multifocal, minimal
83F00005	33463-1	Mononuclear inflammatory cell infiltrate, diffuse, minimal Fibrosis, multifocal, minimal
	33463-2	Mononuclear inflammatory cell infiltrate, diffuse, minimal
83F00006	33464-1	Mononuclear inflammatory cell infiltrate, diffuse, mild Fibrosis, diffuse, mild Epidermal hyperplasia, focally extensive, moderate Folliculitis and furunculosis, focal, moderate
	33464-2	Mononuclear inflammatory cell infiltrate, diffuse, mild
83F00007	33465-1	Mononuclear inflammatory cell infiltrate, multifocal, minimal Fibrosis, focal, minimal Epidermal hyperplasia, focal, minimal
	33465-2	Mononuclear inflammatory cell infiltrate, multifocal, minimal Fibrosis, focal, minimal Epidermal hyperplasia, focal, minimal
83F00009	33467-1	Mononuclear inflammatory cell infiltrate, diffuse, minimal Fibrosis, multifocal, minimal Epidermal hyperplasia, multifocal, minimal
	33467-2	Mononuclear inflammatory cell infiltrate, diffuse, minimal

GLP Study 82003

Acute Dermal Toxicity Study Test (LD_{50}) in Male and Female Rabbits of SEX
Phase I (Limit Test)

TABLE II

Incidence of Microscopic Skin Lesions by Sex and Experimental Group

	MALE		FEMALE	
	Group I	Group II	Group I	Group II
	Saline Control	2 mg/kg	Saline Control	2 mg/kg
Normal Skin	1/5	0/5	0/5	0/5
Infiltration	4/5	2/5	5/5	5/5
Fibrosis	1/5	4/5	3/5	5/5
Hyperplasia	0/5	4/5	4/5	4/5
Dermatitis, pyonecrotizing with ulceration	0/5	0/5	1/5	0/5
Folliculitis with Furunculosis	0/5	0/5	0/5	1/5

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